^{1}H NMR STUDY OF THE CONFORMATION OF THE RIBOSE PHOSPHATE MOIETY OF 6-AZAURIDINE-5′-MONOPHOSPHATE – A NUCLEOTIDE WITH AN UNUSUAL CONFORMATION

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1. Introduction

6-Azauridine-5′-monophosphate (5′-azaUMP, I) is known to be a potent antileukemic agent [1] and to determine whether this activity may be correlated with an unusual conformation, we have examined its molecular geometry in solution using ^{1}H NMR spectroscopy. Comparison of the ^{1}H NMR data on U (uridine), 5′-UMP, 6-azauridine (azaU) and 5′-azaUMP, show that the presence of the 6-aza base has a marked destabilizing influence upon the gauche–gauche (II) conformation constrained to the C(4′) – C(5′) bond of 5′-azaUMP.

Table 1
Coupling constants in Hz for U, 6-azaU, 5′-UMP and 5′-aza-UMP, D_2O solutions, 30°.

Nuclei	U	aza U	5′-UMP	5′-azaUMP
$J_{1'2'}$	4.4	3.6	5.3	4.2
$J_{2'3'}$	5.3	5.2	5.0	5.4
$J_{3'4'}$	5.5	5.8	3.9	5.4
$J_{4'5'}$	3.0	3.2	2.5	4.6
$J_{4'5''}$	4.4	5.6	3.0	6.1
$J_{5'5''}$	−12.7	−12.4	−13.5	−11.3
$J_{1'5}$	0.35	0.61	<0.2	0.55
$J_{P4'}$	–	–	1.8	<0.2
$J_{P5'}$	–	–	3.2	5.6
$J_{P5''}$	–	–	5.1	6.1
$J_{4'5'} + J_{4'5''}$	7.4	8.8	5.5	10.7
$J_{P5'} + J_{P5''}$	–	–	8.3	11.7

2. Materials and methods

Spectra of U, azaU, 5′-UMP and 5′-azaUMP (commercial products), 0.1 M, pD 8.0, 30°, in D_2O, were obtained using either a 220 MHz, continuous wave or a 100 MHz, fast Fourier transform system. Spectra were recorded both with coupling to the ^{31}P of the phosphate and with ^{31}P decoupling and were analyzed using the computer program LAME. In fig. 1 we have illustrated the experimental and computer simulated spectrum (only the ribose region) of 5′-azaUMP. The data are compiled in the table.

3. Results and discussion

Population distribution of the rotamers constrained to the C(4′) – C(5′) bond (II–IV) can be evaluated from the experimental value of $J_{4'5'} + J_{4'5''}$ (Σ) [2, 3]. Any perturbations resulting in an *increase* in the g–g rotamer populations should be manifested by a *decrease* in the experimental *sum* $J_{4'5'} + J_{4'5''}$, whereas an increase in the g–t and/or t–g populations, at the expense of the g–g conformer, should result in an *increase* in the observed sum [2, 3]. Populations distribution of the rotamers constrained to the C(5′) – O(5′) bond (V–VII) can be monitored in an analogous way.

The experimental sum $J_{4'5'} + J_{4'5''}$ (7.4 Hz for U; 8.8 Hz for azaU), shows that the presence of the 6-azauracil base has a destabilizing influence, relative

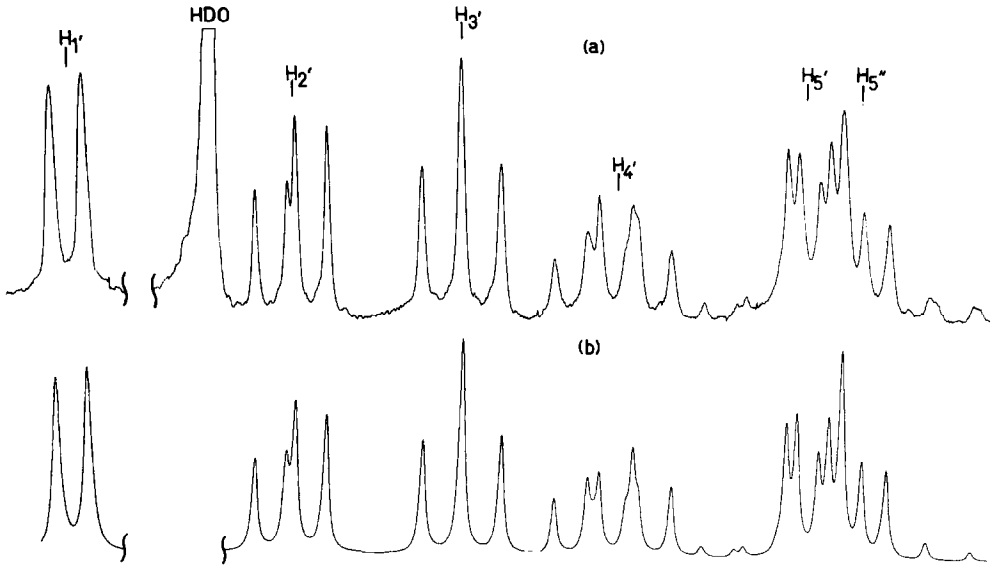


Fig. 1. a) Experimental spectrum of the ribose region of 6-aza-5'-UMP taken in a 100 MHz NMR system, using a 16K fast Fourier transform system. b) Computer simulated spectrum of the ribose region of 6-aza-5'-UMP.

to uracil, upon the g–g orientations (II) about the C(4') – C(5') bond of a nucleoside. This destabilizing effect is more conspicuous for *the monophosphate derivative* for which an *increase* of 5.2 Hz is observed when the uracil is substituted by the 6-azabase. A simple relationship (1) between Pgg (the fractional population of the g–g rotamer) and Σ (the observed sum of $J_{4'5'} + J_{4'5''}$) can be derived by manipulating equations 1–3 in reference 2. This equation provides

$$Pgg \simeq \frac{12 - \Sigma}{8} \qquad (1)$$

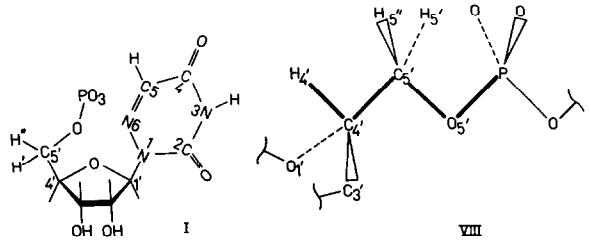
rough estimates of g–g populations. Substitutions of the data into (1) leads to Pgg values: U(60%); azaU (40%); 5'-UMP (80%); 5'-azaUMP (15%). We have derived a similar expression (2) for the C(5') – C(5') bond of the monophosphates

$$Pg'g' \simeq \frac{24 - \Sigma}{18} \qquad (2)$$

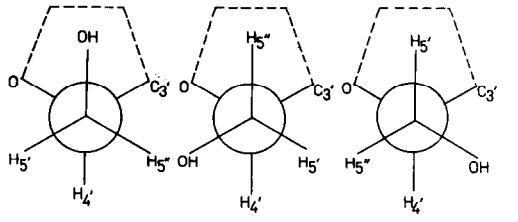
where Pg'g' and Σ are, respectively, the populations of the g'–g' rotamer (V) and the observed sum $J_{P-H(5')} + J_{P-H(5'')}$. The coupling constants used are: $J_{PH\,(gauche)} \simeq 3$ Hz and $J_{PH\,(trans)} = 21$ Hz [4, 5]. The derived Pg'g' values – 5'-UMP (90%), 5'-azaUMP (70%) – suggest a slight destabilizing influence of the 6-aza base upon the g'g' rotamer of a 5'-nucleotide.

In 5'-UMP, but *not* in 5'-azaUMP, we have observed a four bond $J_{P4'}$, long-range coupling. Four bond H–H coupling constants are known to be strongly dependent upon the stereochemical arrangement of the intervening bonds and largest for the all *trans* (W) conformation [6]. Hall and coworkers [4, 7] have presented clear evidence that H–P coupling in H–C–O–P fragments do have a similar stereorelationship. The "W" relationship between the phosphorus and 4' hydrogen of a 5' mononucleotide is encountered when the molecule is simultaneously oriented g–g and g'–g' (VIII), but is destroyed by rotations about either of the C(4') – C(5') or C(5') – O(5') bonds into one of the *trans* conformations. We believe that the observed $^4J_{P4'}$ values for 5'-UMP (1.8 Hz) and 5'-azaUMP (≃0.1 Hz) provide further evidence for the suggested destabilizing influence of a 6-azauracil base upon the g–g and g'–g' conformer of a 5' nucleotide.

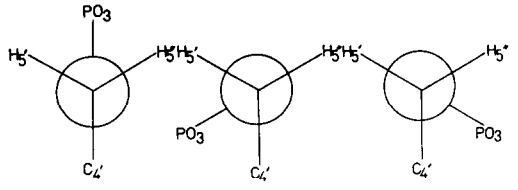
The limitations of the applications of Karplus equation to decipher ribose conformation from $J_{1'2'}$, $J_{2'3'}$ and $J_{3'4'}$ have been discussed elsewhere [8] and it has been suggested that the coupling constant data are best treated in terms of two favored modes viz C(2')



I VIII



GAUCHE-GAUCHE gg II GAUCHE-TRANS gt III TRANS-GAUCHE tg IV



(GAUCHE)'-(GAUCHE)' g'g' V (GAUCHE)'-(TRANS)' g't' VI (TRANS)'-(GAUCHE)' t'g' VII

endo and C(3′) *endo*. The J values in the table indicate that the ribofuranose ring in the nucleosides and nucleotides is not conformationally pure and it may exist as an equilibrium mixture of C(2′) *endo* and C(3′) *endo* modes undergoing interconversion via pseudorotation [9]. If the small differences in the magnitudes of the J values is anything of value, one may indicate that 5′-UMP displays a slight preference for the C(2′) *endo* mode and 5′-azaUMP, a slight preference for the C(3′) *endo* mode.

Comparison of the X-ray data, obtained by Saenger and Suck [10] with the present NMR data, show that 5-azaUMP retains its unusual solution conformation, in the solid state as well, and this is the first time one has encountered a 5′ nucleotide whose exocyclic $CH_2OPO_3^{2-}$ side chain does not follow the general pattern [9a] of g–g, g′–g′ conformation; and it is likely that the antileukemic property of 5′-azaUMP is related to this unusual conformation.

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